Chromatin programming by developmentally regulated transcription factors

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Chromatin programming by developmentally regulated transcription factors: lessons from studying hematopoietic specification and differentiation

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Abstract:

While the body plan of individuals is encoded in their genomes, each cell type expresses a different gene expression program and thus accesses only a subset of this information. Alterations to gene expression programs are the underlying basis for the differentiation of multiple cell types and are driven by tissue-specific transcription factors (TFs) which interact with the epigenetic regulatory machinery to program the chromatin landscape into transcriptionally active and inactive states. The hematopoietic system has long served as a paradigm to study the underlying molecular principles of developmental specific gene expression control. In this review, we will summarize what is known about the mechanism of action of TFs regulating hematopoietic specification and differentiation and we will place this knowledge into the context of general principles of the control of development.

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General principles of the developmental control of gene expression

The control of development involves the successive activation and silencing of specific gene expression programs and thus the selective and cell-type specific use of the transcription machinery which needs to negotiate a chromatin template. Chromatin architecture is an important component of transcription regulation because in the absence of nucleosomes the normal dynamic range of transcription is lost [1]. However, compact chromatin also presents a formidable barrier to the transcription apparatus and requires the concerted action of multiple transcription factors to overcome it [2, 3]. Therefore activation of eukaryotic genes requires an opening up of chromatin structure along an entire genomic domain [4]. There are several ways of performing such a task. One is the passage of a RNA-polymerase complex and its associated factors that splits the two DNA strands as well as dislodges nucleosomes [5, 6]. The other way is via the formation of stable transcription factor (TF) complexes at specific sequences within an active gene domain which interact with each other in 3D space and form an active chromatin hub [7]. Such complexes consist of TFs recognizing specific DNA sequences which, in turn, recruit non-DNA binding co-factors such as chromatin remodellers and modifiers to initiate and maintain an active chromatin state (Figure 1). To understand, how genes are activated and silenced thus driving transitions between cell types during development, regeneration and homeostasis in development, we need to highlight a number of important concepts.

Firstly, an active chromatin structure needs to be constantly maintained. It is now abundantly clear that each transcription cycle is regulated by the balance of activating and repressing factors which ensure that gene expression does not overshoot [8]. In the absence of activators, repressing factors, such as DNA-methyltransferase and histone deacetylases take over, and establish an inactive chromatin structure which is characterised by the absence of TF binding, methylated DNA and deacetylated histones. The second important principle is that an inactive chromatin state is self-perpetuating [9]. This is assured by the deposition of inactive histone marks such as histone H3 lysine 9 methylation (H3K9me3) or H3K27 trimethylation (H3K27me3) which bind silencing factors such as heterochromatin protein 1 (HP1) or Polycomb complexes, which, in turn recruit more co-repressors. Moreover, methylated DNA binds methyl-binding proteins that interact with co-repressors as well. Since DNA methylation is faithfully copied during cell division [10]

repressive complexes are restored and an inactive chromatin structure reforms at inactive genes.

Inactive chromatin is compact, but at the same time highly dynamic. FRAP (fluorescence recovery after photo-bleaching) data employing GFP-tagged histone and chromatin components showed that while nucleosomes tend to bind with a long half-life, transcription factors and non-histone proteins whizz in and out of chromatin complexes within seconds [11, 12] thus presenting windows of opportunities where transcription factors can slip in. Single-molecule imaging studies showed that different TFs patrol the genome and only briefly interact with DNA, but get locked into stable binding once a specific sequence is available for binding [13]. However, there are still open questions as to whether all TFs behave this way and whether all states of closed chromatin permit such highly dynamic scanning movements (such as Polycomb-associated chromatin, as discussed below [14]). Once binding has happened, TFs recruit co-activators, alter chromatin structure from an inactive to an active state, which, in the presence of activators is also maintained through mitosis as some factors can be mitotically retained at their binding sites [15]. In addition, tissue-specific transcription factors cooperate with remodellers to complete with nucleosomes post replication and re-establish nucleosome-free regions [16]. Transcription factors also compete with the DNA-methylation machinery eventually leading to demethylation, either by a passive mechanism or by the active recruitment of demethylase complexes such as TET2 [17, 18]. Depending on which transcriptional regulators are expressed, gene expression patterns are either inherited, thus fulfilling the original definition of epigenetics, or change, driving differentiation. Here we will concentrate on the question of how the chromatin landscape presents itself to transcription factors in a developmental context.

Regulation of the chromatin landscape in development

Many textbooks and PhD theses, which describe gene regulation, tend to start with the statement that we find two types of chromatin in the eukaryotic nucleus: “open” euchromatin and “compact” heterochromatin, containing active and inactive genes, respectively. However, whilst this distinction is convenient, is comes nowhere near the true complexity of dynamic chromatin structure. For example, it is now clear that chromosomes are subdivided into topologically associated domains (TADs) which form the structural backbone of chromatin folding and which are able to partition

chromosomes into regulatory units [19, 20]. In addition, genomic regions associated with the nuclear lamina, so called lamina-associated domains (LADs), are known to represent repressive domains and for some examples it was shown that during development genes move into LADs when being repressed and move out of LADs when being activated [21, 22]. How this intranuclear architecture relates to TADs, however, is poorly understood so far. Moreover, chromatin modifications can be indicative of many different types of transcriptional states [23]. It is outside of the scope of this review to describe the myriad of chromatin proteins and histone modifications that regulate chromatin packaging which are the subject of recent reviews (such as [24]). However, in the context of this article it is relevant that activation and silencing of genes at the level of chromatin structure are gradual processes whereas the regulation of mRNA synthesis tends to be rapid and dynamic [25]. Alterations in transcription factor occupancy and chromatin structure and modifications in development involve the step-wise opening up of chromatin by the sequential assembly of factors in the absence of high-level transcription, a process that is called priming [25, 26]. Conversely, the inactivation of chromatin also occurs gradually, together with the alteration of the transcriptional network within the cell [27]. Transcription can be rapidly switched off which can leave genes in a transitory or poised state ready for further activation of repression, in response to outside signals. This poised state is mediated by Polycomb repressive complexes (PRC) consisting of many different subunits, but involving two types of complexes: PRC1 and PRC2. PRC2 contains a histone modification enzyme (EZH1/2) that deposits one, two or three methyl groups on histone H3K27. The H3K27me3 mark comprises a binding site for PRC1 which then ubiquitinates histone H2A. The result of the formation of Polycomb complexes on promoters and the alteration of chromatin modification is a block of transcriptional elongation by RNA-Polymerase II. However, the non-elongating form of RNA-Polymerase is still binding to Polycomb-repressed promoters [28]. In contrast to the classical consecutive Polycomb-repression model where PRC1 follows PRC2, recent publications also describe a non-canonical class of PRC1 which acts independently of H3K27me3 and where PRC1-mediated H2AK119 ubiquitination recruits PRC2 [29-33].

How does this translate into chromatin architecture? A recent elegant study used FISH combined with super-resolution imaging (STORM) to ask the question of the actual level of compaction of actively transcribed, inactive as defined by a lack of

transcription factor binding and histone modification, and Polycomb-associated poised chromatin within the *Drosophila* nucleus [14]. The choice and classification of gene domains was based on published high-throughput gene expression and chromatin modification data. Bona-fide heterochromatic regions which represent permanently silenced genomic regions [3] such as telomeres or centromers were not analysed. Each of the three classes of chromatin domain had a different level of compaction, with, as expected, transcriptionally active chromatin carrying a high level of histone acetylation having the lowest level of compaction. A surprising result was that transcriptionally inactive and active regions strongly intermingle. In addition, Polycomb-associated chromatin showed the highest level of chromatin compaction which was dependent on the presence of Polycomb as shown by knock-down experiments, and displayed the least intermingling with other types of domains. Polycomb-associated domains showed a very high level of intermixing *within* their domains and behaved like sticky polymers, in line with other experiments in mouse ES cells demonstrating that PRC1 complexes are responsible for promoter-promoter contacts [34]. However, whether Polycomb-bound chromatin always has the highest level of compaction also in mammals is less clear [35]. It is also unclear, how histone modifications, other non-histone proteins and, in vertebrates, DNA-methylation contribute to the compaction status. In any case, these findings highlight the notion that actual chromatin folding is independent from the activity status of the transcription unit. Instead, chromatin architecture is under developmental control determining whether genes are going to be active, have been active or going to be inactive and thus present different targets for transcription factors to negotiate in different cell types.

In the next chapter we will describe hematopoiesis as a model to highlight examples for these principles.

The hematopoietic system as a model to study the developmental control of chromatin programming and gene expression

During embryonic development blood cells emerge from the mesodermal germ layer and their formation occurs temporally and spatially distinctly: the first wave of mouse hematopoiesis takes place in the extra-embryonic blood islands of the yolk sac around day E8.5 and gives rise to primitive progenitor cells with limited potential, mostly erythroid and myeloid [36, 37]. In contrast, the second wave takes place at

day E10.5 at the ventral part of the dorsal aorta in the aorta-gonad-mesonephros (AGM) region of the embryo [38]. Cells emerging during the second wave of hematopoiesis are definitive hematopoietic stem cells (HSCs) that migrate first to the fetal liver and later to the bone marrow where they undergo self-renewal and differentiation throughout lifetime to sustain mature blood cell production. It is believed that a common precursor for hematopoietic and endothelial cells exists, the hemangioblast, which differentiates to a specialised endothelium in the dorsal aorta, the hemogenic endothelium (HE). Cells of the HE undergo an endothelial-to-hematopoietic transition (EHT), during which they detach from the endothelial layer and give rise to intra-aortic clusters of cells comprising HSCs which are then transported to the fetal liver by blood circulation [39-41]. The in vitro differentiation of embryonic stem (ES) cells can be used to recapitulate these developmental transitions and proved to be a powerful tool in order to gain access to rare transient cell populations for studying molecular mechanisms of development [42, 43] (Figure 2A). To this end, pluripotent ES cells are differentiated as embryoid bodies (EBs) and hemangioblast cells are purified based on surface expression of FLK1, i.e. VEGF receptor. Hemangioblast cells can be further differentiated in the presence of BMP4, Activin A and VEGF to smooth muscle, endothelial and hemogenic endothelial cells [39, 44, 45]. Like in the developing embryo, in vitro HE cells undergo an EHT and form hematopoietic progenitor cells which is accompanied by a downregulation of the endothelial program and an upregulation of the hematopoietic program. These developmental pathways are highly conserved in vertebrates and research from a variety of model organisms, including zebrafish and xenopus, contributed to the knowledge we have today [46-48]. Further, immortal hematopoietic precursor cell (HPC) lines have been established from differentiated mouse ES cells, such as the HPC7 line [49].

Using such in vitro models of hematopoietic development helped elucidating the role of developmental stage-specific TFs and subsequent changes of the chromatin landscape [50]. For example, by using knockout ES cell lines it was shown that in the absence of the TF SCL/TAL1 differentiation of hemangioblast to HE cells was blocked, thus highlighting the requirement of SCL/TAL1 for this transition [39, 51]. Further, RUNX1 was found to be essential for the EHT where it activates the hematopoietic program [39, 52], but also a role of RUNX1 was described in HE cells prior to the EHT where it positively regulates cell adhesion genes [53]. GFI1 proteins

were identified as transcriptional repressors needed for silencing of endothelial genes during the EHT [54, 55], while SOX17 proteins were shown to repress hematopoietic genes to maintain the endothelial fate in HE cells [56]. Moreover, several groups mapped open chromatin, histone modifications and TF binding in a genome-wide fashion, revealing common and distinct target genes within highly dynamic transcriptional networks that regulate developmental transitions [42, 57].

Chromatin activation and silencing during hematopoiesis occur in stages

A large number of single gene studies have given us a first insight into how transcription factors assemble during hematopoietic differentiation from lineage-committed progenitors. As already alluded above, also here it was obvious that the activation of individual genes at the level of chromatin remodelling starts way ahead of its high level transcriptional activation. A good example for this notion is the *Csf1r* locus which encodes the receptor for colony-stimulating-factor 1 (CSF1-R), an essential growth factor for macrophage development ([58]). This gene is expressed at a very low level in early hematopoietic progenitor cells, but is strongly up-regulated in committed macrophage precursors. However, even at an early differentiation stage, the gene is occupied by transcription factors and its chromatin is already reorganized and unmethylated. In fact, a number of studies have shown that demethylation of DNA predicts enhancer activity later in development ([59-61]. The up-regulation of the expression of this gene during macrophage differentiation and its response to stimuli is accompanied by the dynamic assembly of different transcription factors [62, 63]. Conversely, the silencing of this gene during B-lymphopoiesis is a gradual process as well, whereby mRNA synthesis ceases first, caused by the silencing of the promoter by the B-cell commitment factor PAX5, followed by a gradual inactivation of chromatin and increased DNA methylation ([64]. Such general behaviour of activation and repression of cis-regulatory elements in alternate lineages, with some lineage-specific variation, has now been multiple times been shown to be true at the genome-wide level (for examples see [42, 65-67].

Development occurs via a series of lineage choices whereby daughter cells eventually start to diversify and switch specific genes on from the silent state. As outlined above, the silent state comes in different flavours and involves various types of chromatin (inactive, repressed, permanently silenced (hetero-)chromatin). How do different types of transcription factors recognise DNA in these different states? This

question is not only important if we want to understand normal development, but also in the context of reprogramming the epigenetic landscape towards pluripotency from somatic cells. As it turns out, life uses multiple mechanisms to solve the problem that most transcription factors have trouble reading the DNA sequence when it is covered by a nucleosome. One mechanism involves a class of transcription factors capable of binding to nucleosomes which mark inactive chromatin for successive activation and successive factor assembly, so called “pioneer factors” the most prominent of which is the winged-helix factor FOXA2 [68, 69]. However, other pioneer factor types, such as EBF1 have also been found [70]. In addition, TFs can recognise partial binding sequences and cooperate to destabilize nucleosomes, followed by the recruitment of histone remodelers and modifiers once a stable complex has formed [71]. It also has been found that the presence of TFs *per se* leads to a gradual increase in DnaseI accessibility at their cognate binding elements via transient binding without forming a stable factor complex. This only forms once all factors participating in such a complex are present [72]. Once such a stable complex is formed, it recruits chromatin remodellers and modifiers that, in the presence of a sufficiently high concentration of participating transcription factors maintain gene expression activity (Figure 1). Recent studies described another hit-and-run mechanism of interactions between transcription factors and chromatin (assisted loading) whereby one type of factor transiently binds to an accessible region in chromatin, recruits chromatin remodellers and leaves in favour of another factor which binds to the same region [73]. In summary, these different studies show clearly that transcription factors cooperate in a highly dynamic and flexible fashion to negotiate chromatin. It is therefore likely that these negotiations will be subtly different for each gene. We still have only a rudimentary understanding of how the cooperation between transcription factors and the epigenetic regulatory machinery ensures that genes are expressed at the right time and the right level [74].

Hematopoietic specification and differentiation is regulated by a relay of transcription factors

The recent years have seen a flurry of genome-wide studies of chromatin alterations during adult hematopoiesis as well as TF binding studies within specific differentiation steps of the hematopoietic hierarchy. A number of studies used ES

cell differentiation to gain insights into the question of how transcription factors regulate embryonic hematopoietic specification [42, 67, 75-78].

The most comprehensive of these studies by Goode et al. [42] mapped both global transcription factor binding and the effects of this binding on chromatin structure and modification over six consecutive stages of hematopoietic specification and differentiation up to the terminally differentiated macrophage stage. The study shows that developmental stage-specific TFs successively become activated and often enhance their own expression as well as the expression of stage-specific genes. In many cases TFs bind to distal regulatory elements of genes before these genes are expressed, a phenomenon called “priming”, whereby in the next differentiation stage these genes acquire promoter histone modifications associated with activation, such as H3K4me3 or H3K9ac, and transcription is initiated. A good example for the gradual process of gene activation during development is the gene encoding the transcriptional repressor GFI1B which is needed during EHT to down-regulate the endothelial program [54, 55]. Gfi1b is not expressed in mesodermal or hemangioblast cells and is expressed at low levels in HE cells. Transcription is highest in HP cells but is then down-regulated and no longer expressed in macrophages. Although the gene is not expressed at the hemangioblast stage, hematopoietic TFs such as SCL, GATA2 and LMO2 bind to *Gfi1b* regulatory regions, while the promoter is still marked by the repressive Polycomb modification H3K27me3 (Figure 2). Subsequently, in HE and HP cells H3K27 methylation is absent, the promoter is marked by H3K4me3 and distal elements are cooperatively bound by a combination of TFs such as GATA1, GATA2, FLI1, ERG, LMO2, RUNX1 and SCL. In macrophages these factors no longer bind to Gfi1b, the promoter re-gains H3K27me3 and transcription is abrogated. This example demonstrates how tissue-specific TFs in a combinatorial fashion program the chromatin template in order to control gene expression at specific developmental stages. However, there is more and more evidence that it is not only tissue-specific TFs alone that perform these programming events, but in many developmental contexts ubiquitously expressed and signalling-inducible TFs cooperate with them. For example, from motif analyses of DNA sequences within DNaseI hypersensitive, i.e. chromatin sites which are accessible due to TF binding it could be shown that the Hippo-signalling-sensitive TF TEAD4 binds to sites occupied by tissue-specific TFs such as FLI1 and SCL/TAL1 during early hematopoietic specification and that binding and the

interaction with its co-factor YAP is essential for the generation of hematopoietic cells *in vitro* [42]. Further, in the absence of the ubiquitous TF SP1 during early embryonic hematopoiesis, later stages of differentiation are severely affected, highlighting the role of such factors for tissue-specific gene regulation and chromatin programming [76].

While some TFs are known for their potential to activate gene expression, others have repressive potential during hematopoietic specification. Examples are GFI1/GFI1B proteins which repress the endothelial program during the EHT [54, 55], or SOX17 which represses hematopoietic genes in HE cells [56]. However, it remains a challenge for researchers to understand how exactly some TFs at defined genomic elements, in combination with specific other factors, at distinct developmental stages can act either as activators or repressors. The induction of RUNX1 during the EHT results in a vast number of genes being bound by RUNX1, and while most of them are transcriptionally activated, some others are down-regulated, e.g. Sox17 [75]. Also SCL/TAL1, PAX5 and EBF1 were shown to have a context-dependent activating or repressive effect on gene expression [79-82]. It is therefore likely that the nature of TF assemblies determines a context-dependent recruitment of activating or repressive members of the epigenetic regulatory machinery.

The epigenetic regulatory machinery modifying transcription factors and chromatin components is essential for hematopoietic development

A variety of regulatory mechanisms can be used by a cell to regulate TF activities such as modulating transcription of the TF gene itself, post-translational modifications that allow or prevent nuclear localisation, and post-translational modifications that affect the protein stability and activity. Second, TFs often act together with other TFs and for some genes spatial and temporal co-expression of a set of co-operative TFs and their co-binding is a prerequisite for transcriptional initiation. Third, TFs recruit enzymatic co-factors which remodel and modify the chromatin template, make it more accessible and in case of promoter-enhancer looping support the assembly and initiation of the transcriptional machinery at the target promoters.

An example of a TF that can be subject to modifications itself and that recruits chromatin modifiers is RUNX1. Depending on cell type and genomic region, RUNX1

was reported to interact with a multitude of different modifiers such as histone acetyltransferases [83, 84] and the nucleosome remodeller SWI/SNF [85] that support the activating role of RUNX1. However, RUNX1 can also recruit repressive complexes like Suv39H1 [86], mSin3A [87] or Polycomb group proteins [88] as well as histone deacetylases [89] reflecting that it can also act as a repressor of transcription. Further, it has been shown that MAPK signalling via ERK can causes phosphorylation of the RUNX1 protein which subsequently leads to the disaggregation of a complex that RUNX1 can form with the repressor mSin3A, thus removing repressive potential from the TF and enhancing its activating roles [87]. Moreover, the histone acetyltransferases p300/CBP and MOZ can have a dual role when forming a complex with RUNX1: i) recruited by RUNX1 these enzymes boost histone acetylation of the nearby nucleosomes, help opening up the chromatin fibre and provide a binding platform for other activators containing bromodomains, ii) p300/CBP and MOZ can use RUNX1 itself as their substrate and thus increase its transactivation potential [83, 84]. In the light of this complexity it is not surprising that mutations, deletions or translocations affecting chromatin modifiers can lead to disturbed differentiation and cancer [90]. However, the molecular mechanisms of how such mutations derail normal differentiation are still largely unknown and a matter of extensive current research.

Conclusions and Perspectives

The interaction of transcription factors with the chromatin landscape comprises a robust, but also highly flexible and dynamic system geared towards a regulated response to outside signals and developmental cues. Intermolecular interactions are at the heart of these processes and these interactions are specific for each gene. We are slowly approaching the point at which we can begin to construct transcriptional networks allowing predictions of the transcriptional response of individual cell types to changing TF expression levels [91]. However, the integration of the chromatin landscape as a dynamic modulator of the transcriptional response of differentiating cells into such models presents a formidable problem whose solution is still in its infancy.

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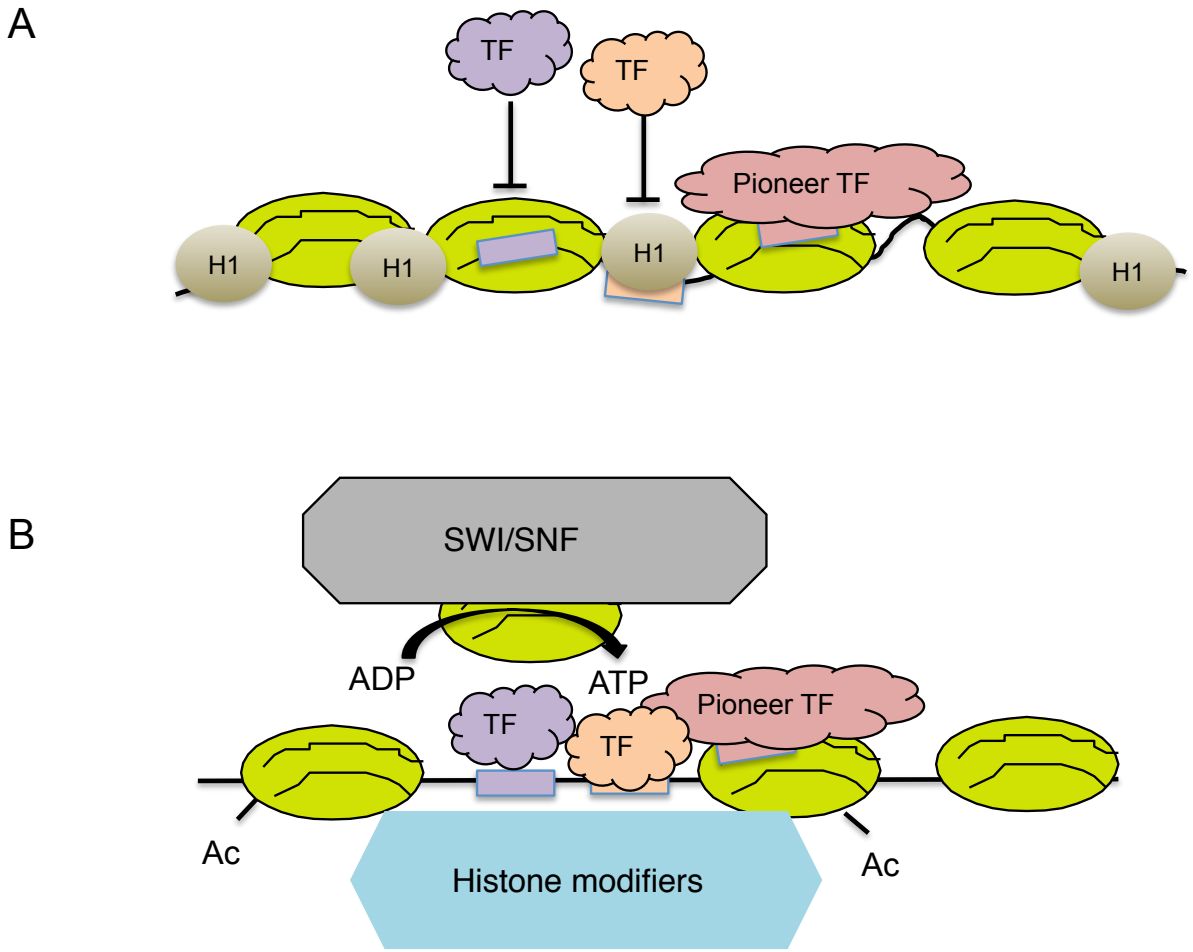

Figure 1: The interaction of transcription factors with chromatin

(A) Simplified scheme how different ways how transcription factors negotiate the chromatin landscape. Transcription factor binding motifs which are placed at different position relative to nucleosomes are depicted as coloured rectangles. TF: transcription factors which have different properties depending on whether they can bind to nucleosomal DNA or not. H1: histone H1 (B) Once a stable transcription factor complex has formed, SWI/SNF-type complexes evict nucleosomes and facilitate TF binding and co-activator (histone modifier) recruitment. Ac: Histone acetylation

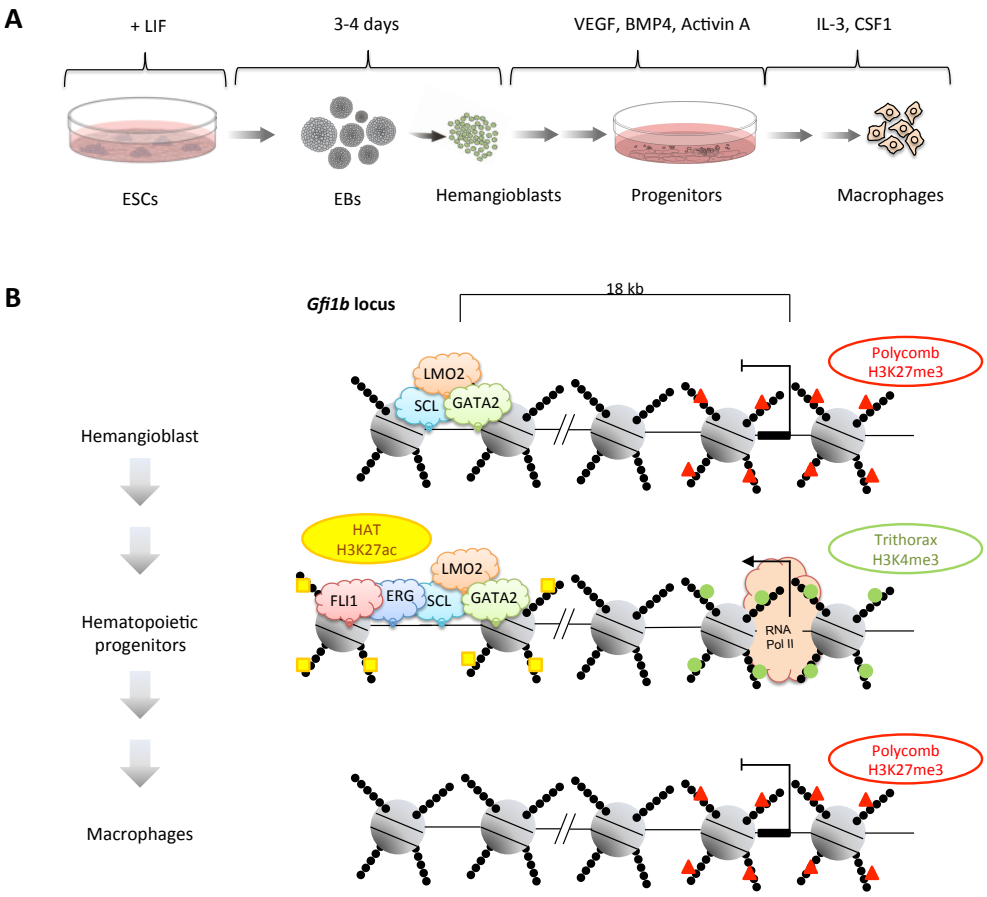

Figure 2: The developmental activation and deactivation of the *Gfi1b* locus

(A) Scheme depicting the differentiation of hemangioblasts, hematopoietic progenitors and macrophages from differentiated mouse embryonic stem cells. (B) Assembly of transcription factors and histone modifications at the *Gfi1b* locus (modified from Goode et al; 2016)